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Modulation of murine T and B lymphocyte subsets by polysaccharide fraction B isolated from *Caltha palustris L.*

AGNIESZKA SUSZKO[1], MARIANNA SZCZYPKA[1], MAGDALENA LIS[1], JANINA KUDUK-JAWORSKA[2], BOŻENA OBMIŃSKA-MRUKOWICZ[1]

[1]Department of Biochemistry, Pharmacology and Toxicology, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, Poland
[2]Institute of Chemistry, Wrocław University, Poland

Abstract

Various natural plant extracts have been shown to have the immunomodulatory activity. Caltha palustris L. (Ranunculaceae) is a plant that is widely known and distributed in Europe, Asia and North America. The extracts from Caltha palustris have been used in traditional Canadian and Asian medicine to treat arthritis rheumatism, gonorrhea and a variety of skin diseases. The effects of polysaccharide fraction B from Caltha palustris L. extract (0.1, 1 and 10 mg/kg) on the total number of lymphocytes in the thymus, spleen and mesenteric lymph nodes and the percentage and absolute number of T cells ($CD4^-CD8^-$, $CD4^+CD8^+$, $CD4^+$, $CD8^+$) in the thymus and T cells ($CD3^+$, $CD4^+$, $CD8^+$) and B ($CD19^+$) lymphocytes in the spleen and mesenteric lymph nodes in mice were studied. The investigated substance was administered intraperitoneally once or five times to mice. The measurements were determined twice: 24 and 72 h after the last administration of fraction B. It was found that five times administration of fraction B (0.1 mg/kg) from Caltha palustris extract significantly decreased the absolute count of $CD8^+$ thymic cells on both days. The increase in the $CD4^+$ lymphocyte population (thymus, spleen) after single administration of fraction B from Caltha palustris extract was noticed, however a decrease in this population was observed after the multiple administration in mesenteric lymph nodes. Whereas $CD19^+$ B lymphocyte population of spleen has been stimulated after five times administration of the tested agent. Results of the study demonstrated that fraction B from Caltha palustris extract can change the percentage and absolute number of T and B lymphocytes in lymphatic organs. The effect of the examined substance depends on the number of consecutive doses applied.

Key words: *polysaccharide, Caltha palustris L., extract, B and T lymphocyte subsets, mice.*

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Introduction

Natural plant extracts can be an alternative and reliable source for the development of new drugs [1–3]. Various plant extracts used in traditional medicine for millennia have been shown to possess nonspecific immunomodulating properties and natural remedies derived from *Aloe vera* might serve as an example [4]. There are many commercial formulations on the basis of Aloe vera plant but their immunomodulatory activities are still under investigation [5]. *Caltha palustris* L. (Ranunculaceae) is a plant that is widely known and affluently distributed in Europe, Asia and North America. The extracts obtained from *Caltha palustris* have been used in traditional medicine: it has been used to treat arthritis [6] leprosy, rheumatism, gonorrhea [7], and a variety of skin lesions [8]. Sokoloff's studies demonstrated that a water extract of Caltha palustris shows slight oncostatic activity against some mice tumors (Sarcoma S-180, Ehrlich carcinoma) [9]. Isolation of the polysaccharide fractions of the investigated plant, their chemical and physical properties and some of their biological effects, which were studied *in vitro* were described. It was

Correspondence: Bożena Obmińska-Mrukowicz, Department of Biochemistry, Pharmacology and Toxicology, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, Norwida 31, 50-375 Wrocław, Poland, e-mail: b.mrukowicz@gmail.com

presented that fraction B suppressed both lymphocyte and granulocyte activation and diminished the proliferation of human leukemic cell cultures. Fraction B from *Caltha palustris* L. extract inhibited *in vitro* proliferative activity of PHA-induced human lymphocyte, and decreased the granulocytes H_2O_2 production and release in the cultures without the addition of PMA [10, 11].

In this study we examined and characterized the immunomodulatory effect of polysaccharide fraction B of *Caltha palustris* extract on T cell subsets in thymus and T and B lymphocytes in the spleen and mesenteric lymph nodes. Our previous experiments focused on fraction C of *Caltha palustris* extract [12].

Material and methods

Plant material and preparation of polysaccharide fraction

The plant material was collected near Trzebnica (West Poland). The dried, powdered material (1000 g) was extracted with methanol (5L) for 48 h and afterwards, the solution was discarded. After evaporation of the dissolvent, the residual herb was extracted with distilled water to obtain water extract (A) and residue. Then, the residue was extracted with 0.1 M NaOH to obtain extract B. Extract B was reduced *in vacuo* to 2L without heating. Next, it was centrifuged and the supernatant was treated with TCA and then with acetone. Fraction B, obtained from 0.1 M NaOH extract tested positive for saccharides in reaction with phenol reagent [13] and was found to contain polysaccharides of molecular weight 3.4×10^4 Da and 3.2×10^4 Da (Biogel-P10 – gel permeation chromatography). Total sugar content (22%) was determined as anhydroglucose after Dubois *et al.* [13], uronic acids (2.8%) by the Blumenkrantz method [14]. The investigated fraction also contained a significant quantity of ash – 47%. A stock solution of the plant extract fraction was prepared *ex tempore* by dissolving 2 mg of fraction B in phosphate-buffered saline solution (PBS, Institute of Immunology and Experimental Therapy, Wrocław, Poland).

Animals

The studies were conducted on Balb/c mice (8-10 weeks of age; male and female), each weighing 20-22 g. The mice were obtained from the Breeding Center of Laboratory Animals at the Institute of Occupational Medicine, Łódź, Poland. The animals were kept under the conventional conditions. The mice were fed commercial granulated food and water *ad libitum*. The study protocol was approved by the Local Ethics Committee (No. 22/2008).

Drugs and treatment

The solution of fraction B from *Caltha palustris* extract at doses of 10, 1, and 0.1 mg/kg, was administered intraperitoneally (i.p.), once or five times at 24 h intervals. The volume of each dose was 0.2 ml per animal. The trials in the control group were conducted simultaneously. The mice in the control group received phosphate buffered saline – PBS (0.2 ml/mouse). Each control and experimental group consisted of seven mice.

Measurements

The measurements were determined at two time points: 24 h (day 1) and 72 h (day 3) after the last administration of fraction B from *Caltha palustris* extract.

The measurements included: (a) the total number of thymocytes, splenocytes and lymphocytes of the mesenteric lymph nodes, (b) the weight ratio of the thymus, spleen, mesenteric lymph nodes calculated according to the following formula: weight of organ (g)/body weight of mouse (g) × 100 and (c) the percentage and count of CD subsets ($CD4^-CD8^-$, $CD4^+CD8^+$, $CD4^+$, $CD8^+$ in thymus, $CD19^+$, $CD3^+$, $CD4^+$, $CD8^+$ in spleen and mesenteric lymph nodes).

Assay of thymocyte, splenocyte and lymphocyte of mesenteric lymph node subpopulations

The mice were anesthetized with halothane (Narcotan, Zentiva, Prague, Czech Republic), and killed by cervical dislocation 24 h and 72 h after the last administration of fraction B from *Caltha palustris* extract. Thymus, spleens and mesenteric lymph nodes were removed and placed in disposable Petri dishes containing sterile ice-cold PBS. The suspended cells were removed from the lymphatic organs by gentle passage through a nylon mesh and then centrifuged (2250 × g, 15 min, 4°C) on a layer of Ficoll 400 (Pharmacia, Fiene Chemicals AB, Sweden)/Uropolinum 75% (diatrizoate sodium and meglumine diatrizoate; Polpharma, Poland) at a 1 : 3 ratio and density of 1.071. After centrifugation, the cells were collected from the interphase and washed twice (375 × g, 8 min, 4°C) with PBS supplemented with 1% bovine serum albumin (BSA, Sigma, USA) at 4°C. After the second wash, the cells were resuspended in PBS with 1% BSA at 1×10^7 cells/ml. The viability of each cell suspension, as determined by trypan blue dye exclusion, was 90-95%. The cells were resuspended in 100 µl of PBS solution containing 1% BSA. The thymocytes, splenocytes and lymphocytes of mesenteric lymph nodes were stained with the monoclonal antibodies: Rat Anti Mouse CD4:FITC/CD8:RPE dual color reagent (Serotec, Kidlington, UK), at the dilutions recommended by the manufacturer. The splenocytes and lymphocytes of the mesenteric lymph nodes were also stained with the antibodies: Rat Anti Mouse CD19:FITC/CD3:RPE dual color reagent (Serotec, Kidlington, UK), according to the manufacturer's instructions. Cells were incubated at 4°C for 30 min and then washed 3 times with ice-cold PBS. Fluorescence was analyzed using a flow cytometer (FACS Calibur; Becton

Table 1. The weight ratio of lymphatic organs and total number of thymocytes, splenocytes and lymphocytes in mesenteric lymph nodes in mice treated once (A) and five times (B) with fraction B of *Caltha palustris* extract. The mean values (n = 7) and standard deviations are presented

A

Index	Day	Control	Fraction B of *Caltha palustris* extract		
			1 × 10 mg/kg	1 × 1 mg/kg	1 × 0.1 mg/kg
weight ratio of thymus	1	0.182 ±0.02	0.176 ±0.041	0.144 ±0.024*	0.175 ±0.039
	3	0.246 ±0.07	0.167 ±0.039*	0.228 ±0.082	0.207 ±0.059
total number of thymocytes (× 10^6)	1	16.5 ±5.22	35.52 ±13.02*	43.28 ±22.72*	69.20 ±20.44*
	3	47.43 ±13.09	31.11 ±5.24*	32.31 ±5.14*	26.34 ±4.09*
weight ratio of spleen	1	0.583 ±0.09	0.644 ±0.08	0.660 ±0.07	0.569 ±0.09
	3	0.565 ±0.10	0.745 ±0.10*	0.688 ±0.08*	0.740 ±0.19*
total number of splenocytes (× 10^6)	1	143.04 ±54.17	103.07 ±43.63	141.67 ±44.78	124.86 ±23.08
	3	63.14 ±4.34	64.00 ±11.99	54.66 ±6.38*	59.49 ±7.91
weight ratio of mesenteric lymph node	1	0.481 ±0.09	0.471 ±0.06	0.443 ±0.09	0.422 ±0.04
	3	0.466 ±0.10	0.500 ±0.10	0.502 ±0.09	0528 ±0.04
total number of lymphocytes in mesenteric lymph nodes (× 10^6)	1	4.80 ±1.66	10.80 ±8.02	7.40 ±3.72	10.93 ±3.85*
	3	18.19 ±3.55	16.16 ±2.88	12.73 ±1.55*	16.59 ±1.86

B

Index	Day	Control	Fraction B of *Caltha palustris* extract		
			5 × 10 mg/kg	5 × 1 mg/kg	5 × 0.1 mg/kg
weight ratio of thymus	1	0.500 ±0.08	0.581 ±0.08	0.551 ±0.07	0.511 ±0.024
	3	0.531 ±0.05	0.732 ±0.28	0.662 ±0.09	0.642 ±0.19
total number of thymocytes (× 10^6)	1	70.33 ±14.07	65.89 ±26.66	47.07 ±18.20*	77.60 ±34.43
	3	65.67 ±10.59	64.69 ±13.13	70.40 ±14.40	92.06 ±21.01*
weight ratio of spleen	1	0.500 ±0.08	0.581 ±0.08	0.551 ±0.07	0.511 ±0.024
	3	0.531 ±0.05	0.732 ±0.28	0.662 ±0.09	0.642 ±0.19
total number of splenocytes (× 10^6)	1	70.33 ±14.07	65.89 ±26.66	47.07 ±18.20*	77.60 ±34.43
	3	65.67 ±10.59	64.69 ±13.13	70.40 ±14.40	92.06 ±21.01*
weight ratio of mesenteric lymph node	1	0.351 ±0.07	0.371 ±0.05	0.401 ±0.09	0.393 ±0.06
	3	0.472 ±0.15	0.471 ±0.07	0.491 ±0.06	0.482 ±0.17
total number of lymphocytes in (× 10^6) mesenteric lymph nodes	1	11.4 ±2.82	4.40 ±2.44*	5.20 ±1.63*	5.94 ±2.01*
	3	17.40 ±7.73	9.13 ±3.26*	6.24 ±1.37*	7.27 ±4.20

**p < 0.05 as compared to the control group*

Dickinson, Germany). Data acquisition and analysis were done using the Cell Quest 3.1f software.

Statistical analysis

Student's *t*-test for independent samples at a significance level of 5% was used to compare the efficacy of treatment. In each case, the differences between the mean values of the control group versus treated group (fraction B at a dose of 1 × 0.1 mg/kg and 1 × 1 mg/kg and 1 × 10 mg/kg, and at a dose of 5 × 0.1 mg/kg and 5 × 1 mg/kg and 5 × 10 mg/kg, respectively, after 24 h and 72 h) were studied. The calculations were made using the Statistica 9.1 StatSoft package.

Results

As Table 2A shows, a single administration of fraction B from *Caltha palustris* extract at doses 1 and 0.1 mg/kg temporarily but significantly increased the absolute count of mature $CD8^+$ thymic cells, measured on day 1. In turn, 3 days after a single administration of the tested fraction, the significant decrease in the absolute count of $CD8^+$ cells in thymus at all three doses (0.1 mg/kg, 1 mg/kg, 10 mg/kg) was found. Further, it was followed by the decrease in the absolute count of $CD4^+CD8^+$ thymocytes (double-positive cells) and accompanied by the decrease in the total number of thymocytes, at all investigated doses. The increase in the absolute count of $CD4^+$ thymic cells was observed at all three doses (0.1 mg/kg, 1 mg/kg, 10 mg/kg), only 24 h after a single injection. However, a temporarily increase in the absolute count of immature $CD4^-CD8^-$ (double-negative cells) thymocytes 24 h after a single administration was noticed. Five times (Table 2B) administration of fraction B (0.1 mg/kg) from *Caltha palustris* extract significantly decreased the absolute count of $CD8^+$ cells on both days. Moreover, the decrease in the absolute count of $CD4^+CD8^+$ cells was observed after the multiple administration of polysaccharide fraction B at a dose of 0.1 mg/kg measured on days 1 and 3 and 10 mg/kg on day 1. Additionally, the decrease in the percentage and absolute count was noticed after five exposures to the tested agent (0.1 mg/kg) measured on both days. After five injections of fraction B, the decrease in the total number of thymocytes was observed at a dose of 0.1 mg/kg on both days and at a dose of 1 mg/kg on day 1. As Table 3A shows, a single administration of fraction B at a dose of 0.1 mg/kg increased the percentage of $CD3^+$ (Pan-T cells) (day 1 – dose 1 mg/kg; day 3 – all 3 tested doses), which is followed by the increase in the percentage of $CD4^+$ splenocytes. Multiple administration of fraction B (Table 3B) at a dose of 0.1 mg/kg increased the percentage of $CD19^+$ (B lymphocytes) in the spleen on both days, whereas a single administration exerted the opposite effect, but only on day 3. However, five exposures to fraction B at a dose of 1 mg/kg and 0.1 mg/kg decreased the percentage of $CD3^+$ cells accompanied by the decrease in the percentage $CD8^+$ splenocytes on both days.

A single administration of investigated fraction B (0.1 mg/kg, 1 mg/kg, 10 mg/kg) caused the decrease in the percentage of B lymphocytes ($CD19^+$) from mesenteric lymph nodes determined on day 1 and the decrease in their absolute count after administration at doses of 0.1 mg/kg, 1 mg/kg, measured on day 3 (Table 4A). Moreover, a single injection of fraction B resulted in the enhanced percentage of $CD3^+$ cells (0.1 mg/kg, 1 mg/kg, 10 mg/kg) on day 1, accompanied by the increase in the percentage of $CD4^+$ mesenteric lymph nodes lymphocytes population on day 1 (10 mg/kg, 1 mg/kg). Five exposures to fraction B (Table 4B) decreased temporarily the absolute count of $CD19^+$ cells on day 1 (0.1 mg/kg, 1 mg/kg). Multiple injections of fraction B resulted in the decrease in the percentage and absolute count (day 3) of $CD3^+$ cells and only the percentage (day 1) at all doses applied. Furthermore, the decrease in the absolute count of $CD8^+$ cells measured on both days was observed, at all 3 tested doses.

Discussion

In the present study, we confirmed immunomodulating activities of polysaccharide fraction B from *Caltha palustris* extract. Many polysaccharide compounds in other plants (such as *Solanum nigrum*, *Opilia celtidifolia*, *Potentilla anserina*) have been reported to have immunomodulating properties [15-17]. The most meaningful parameters for evaluating the balanced state of immunomodulation and homeostatic responses of the intrinsic immune system are the number and ratio of two main lymphocyte T subsets ($CD4^+$ cells – T helpers, and $CD8^+$ cells – T cytotoxic/suppressors) [18]. The recognition of antigens presented by the major histocompatibility complex class II (MHC II) proteins and mediation of both cellular immune responses through Th1 cells and humoral immune responses through Th2 cells are the domain of $CD4^+$ T cells. The $CD8^+$ cells recognize antigens presented by MHC I molecules and mediate cellular immune responses through cytotoxic T cells. Wu *et al.* [19], who investigated the traditional Chinese medicine, Chi-Shie-Shuang-Bu-An-Shen-Tang (CST), reported the significant increase in the population of CD4+ T cells in spleen after oral administration of sterilized CST for 3 weeks. The authors suggest that probably the polysaccharide component was responsible for this activity. In the present study, the increase in the CD4+ lymphocyte population (thymus, spleen) after a single administration of fraction B from *Caltha palustris* extract was noticed, but on the other hand, the decrease in this population was observed after the multiple administration in mesenteric lymph nodes. According to the data obtained in the cited study, the increase in $CD4^+$ population may be potentially beneficial, especially in potential treatment of the states of $CD4^+$ lymphopenia characteristic of systemic diseases, i.e. systemic lupus erythromatosus [20] or action of drugs, i.e. cyclophosphamide [21] may increase the risk of infection. However, the decrease noticed in mesenteric lymph nodes after a longer exposure to the tested fraction might be problematic.

$CD8^+$ and $CD4^+$ subpopulations, which in peripheral blood, play also a crucial role in adult host defense toward cancer [22]. The fraction 1a of polysaccharides, isolated from *Solanum nigrum* Linne (SNL-P1a) has been demonstrated to have a protective effect on thymus in tumor-bearing mice. Li *et al.* [15] studied the profile of $CD4^+$ and $CD8^+$ in PBMC (peripheral blood mononuclear cell). The authors showed that treatment with SNL-P1a following tumor implantation caused a significant increase in the number of $CD4^+$ T-lymphocyte and a decrease in the number of $CD8^+$

Table 2. Percentage and absolute count of thymocyte subpopulations in mice treated once (A) and five times (B) with fraction B of *Caltha palustris* extract. The mean values (n = 7) and standard deviations are presented

A

Index		Day	Control	Fraction B of *Caltha palustris* extract		
				1 × 10 mg/kg	1 × 1 mg/kg	1 × 0.1 mg/kg
$CD4^-CD8^-$	(%)	1	4.27 ±0.98	4.47 ±1.08	4.02 ±1.00	4.34 ±0.89
	($\times 10^6$)		0.61 ±0.17	1.53 ±0.42*	1.87 ±1.03*	2.97 ±0.88*
	(%)	3	3.29 ±2.48	3.06 ±0.86	2.17 ±0.59	2.92 ±0.63
	($\times 10^6$)		1.44 ±0.72	0.93 ±0.21	0.70 ±0.25*	0.76 ±0.15*
$CD4^+CD8^+$	(%)	1	73.53 ±2.69	68.99 ±1.75*	73.74 ±2.85	73.26 ±3.22
	($\times 10^6$)		12.47 ±4.16	24.48 ±9.45	40.61 ±11.80*	50.36 ±15.42*
	(%)	3	82.76 ±3.48	80.65 ±2.52	83.69 ±2.18	81.38 ±1.97
	($\times 10^6$)		39.61 ±11.86	25.14 ±4.57*	26.86 ±4.27*	21.49 ±3.72*
$CD4^+$	(%)	1	18.88 ±1.79	21.99 ±1.02*	18.81 ±1.86	18.99 ±1.97
	($\times 10^6$)		2.91 ±0.81	8.44 ±2.77*	8.59 ±5.39*	13.45 ±4.11*
	(%)	3	9.12 ±1.53	11.81 ±2.06	10.06 ±1.12	11.69 ±0.99
	($\times 10^6$)		4.16 ±1.30	3.66 ±0.80	3.28 ±0.93	3.06 ±0.43*
$CD8^+$	(%)	1	3.33 ±0.47	4.60 ±0.58*	3.43 ±0.56	3.41 ±0.6
	($\times 10^6$)		0.51 ±0.16	1.63 ±0.62	1.62 ±1.09*	2.41 ±0.64*
	(%)	3	4.90 ±0.85	4.48 ±0.75	4.04 ±0.98	4.02 ±0.9
	($\times 10^6$)		2.27 ±0.63	1.38 ±0.26*	1.32 ±0.46*	1.04 ±0.16*

B

Index		Day	Control	Fraction B of *Caltha palustris* extract		
				5 × 10 mg/kg	5 × 1 mg/kg	5 × 0.1 mg/kg
$CD4^-CD8^-$	(%)	1	7.71 ±2.52	10.64 ±3.95	5.96 ±0.93	4.08 ±0.73*
	($\times 10^6$)		2.08 ±0.42	1.82 ±0.32	1.46 ±0.59	0.49 ±0.06*
	(%)	3	3.48 ±0.67	4.17 ±1.69	4.15 ±0.87	4.12 ±1.34
	($\times 10^6$)		1.44 ±0.48	1.12 ±0.19	1.00 ±0.30*	0.90 ±0.43*
$CD4^+CD8^+$	%	1	73.46 ±5.90	67.71 ±4.62	77.18 ±3.34	79.85 ±2.11
	($\times 10^6$)		26.28 ±10.14	13.59 ±5.81*	20.15 ±8.41	10.70 ±2.89*
	(%)	3	78.13 ±3.87	78.28 ±3.07	75.65 ±3.73	78.89 ±3.91
	($\times 10^6$)		33.18 ±12.58	33.83 ±11.71	18.95 ±0.34*	19.65 ±8.38*
$CD4^+$	(%)	1	13.40 ±1.11	17.30 ±1.32*	14.08 ±2.08	13.35 ±1.64
	($\times 10^6$)		5.00 ±1.91	3.48 ±1.62	3.53 ±1.59	1.79 ±0.40*
	(%)	3	15.67 ±3.36	14.82 ±1.82	15.95 ±1.83	14.66 ±2.35
	($\times 10^6$)		6.38 ±1.98	5.92 ±1.91	4.00 ±1.21	3.34 ±1.26*
$CD8^+$	(%)	1	2.54 ±0.84	4.35 ±0.19*	2.78 ±0.74	2.72 ±0.41
	($\times 10^6$)		0.79 ±0.29	0.81 ±0.36	0.62 ±0.16	0.33 ±0.07*
	(%)	3	2.58 ±0.33	2.73 ±0.39	2.83 ±0.52	2.33 ±0.64
	($\times 10^6$)		1.08 ±038	1.10 ±0.49	0.70±0.30	0.64 ±0.41*

**p < 0.05 as compared to the control group*

Table 3. Percentage and absolute count of splenocyte subpopulations in mice treated once (A) and five times (B) with fraction B of *Caltha palustris* extract. The mean values (n = 7) and standard deviations are presented.

A

Index		Day	Control	Fraction B of *Caltha palustris* extract		
				1 × 10 mg/kg	1 × 1 mg/kg	1 × 0.1 mg/kg
$CD3^+$	(%)	1	39.87 ±5.99	43.46 ±2.95	45.70 ±2.90*	41.18 ±6.25
	($\times 10^6$)		56.35 ±21.30	44.56 ±19.63	64.73 ±22.43	51.41 ±12.05
	(%)	3	33.13 ±3.25	38.70 ±1.68*	36.42 ±2.75*	39.98 ±4.71*
	($\times 10^6$)		21.03 ±3.39	24.82 ±4.88	19.77 ±1.18	23.78 ±4.16
$CD4^+$	(%)	1	28.27 ±3.62	30.09 ±2.34	33.08 ±3.28*	31.16 ±4.36
	($\times 10^6$)		40.14 ±15.19	26.38 ±16.80	40.16 ±23.55	39.05 ±9.60
	(%)	3	23.7 ±3.79	28.40 ±2.38*	29.31 ±4.02*	29.69 ±3.15*
	($\times 10^6$)		14.69 ±3.32	18.19 ±3.67*	15.22 ±2.63	14.65 ±6.69
$CD8^+$	(%)	1	7.55 ±1.51	9.11 ±0.63*	8.57 ±1.56	8.83 ±2.67
	($\times 10^6$)		7.43 ±6.01	8.24 ±5.52	10.73 ±7.13	10.93 ±3.57
	(%)	3	5.05 ±1.02	6.67 ±0.71	6.92 ±1.53	7.11 ±0.94
	($\times 10^6$)		3.21 ±0.81	4.26 ±0.90*	3.53 ±0.91	4.18 ±0.48*
$CD19^+$	(%)	1	55.82 ±6.33	51.07 ±2.81	48.79 ±2.92*	52.27 ±6.40
	($\times 10^6$)		80.63 ±33.27	53.03 ±22.78	69.24 ±20.81	65.47 ±16.04
	(%)	3	57.42 ±3.48	53.08 ±2.24	58.37 ±5.58	52.70 ±3.97*
	($\times 10^6$)		36.14 ±1.05	34.00 ±6.87	32.12 ±6.21	31.34 ±4.88*

B

Index		Day	Control	Fraction B of *Caltha palustris* extract		
				5 × 10 mg/kg	5 × 1 mg/kg	5 × 0.1 mg/kg
$CD3^+$	(%)	1	49.52 ±2.16	45.81 ±8.70	37.63 ±2.66*	34.27 ±4.71*
	($\times 10^6$)		34.93 ±6.02	34.85 ±14.80	18.05 ±7.89*	25.70 ±12.06
	(%)	3	38.93 ±7.20	38.35 ±3.91	31.77 ±6.91*	30.40 ±7.19*
	($\times 10^6$)		25.55 ±5.81	25.04 ±6.78	22.40 ±6.17	27.20 ±5.94
$CD4^+$	(%)	1	27.18 ±5.00	32.45 ±5.68*	27.47 ±4.09	25.83 ±3.42
	($\times 10^6$)		19.27 ±3.15	22.04 ±10.72	12.14 ±6.07*	19.49 ±9.17
	(%)	3	28.75 ±2.81	30.42 ±2.26	27.84 ±5.42	24.47 ±5.91
	($\times 10^6$)		18.77 ±3.24	19.79 ±4.88	19.51 ±4.87	21.84 ±4.66
$CD8^+$	(%)	1	8.33 ±1.86	7.84 ±2.17*	5.13 ±1.32*	5.95 ±1.87*
	($\times 10^6$)		6.10 ±1.64	5.45 ±3.02	2.70 ±1.47*	4.25 ±2.36
	(%)	3	6.66 ±1.03	7.09 ±0.90	4.66 ±1.47*	4.55 ±1.15*
	($\times 10^6$)		4.36 ±1.02	4.64 ±1.32	3.25 ±1.13	4.09 ±1.05
$CD19^+$	(%)	1	44.37 ±2.19	47.28 ±8.68	54.73 ±3.12*	57.84 ±4.87*
	($\times 10^6$)		31.12 ±7.21	29.96 ±9.50	25.30 ±9.00	45.99 ±20.55
	(%)	3	55.95 ±7.26	54.93 ±4.72	60.86 ±7.65	63.08 ±7.45*
	($\times 10^6$)		36.71 ±8.70	35.29 ±6.22	42.69 ±9.90	58.90 ±18.08

$p < 0.05$ as compared to the control group

Table 4. Percentage and absolute count of mesenteric lymph node cell subpopulations in mice treated once (A) and five times (B) with fraction B of *Caltha palustris* extract. The mean values (n = 7) and standard deviations are presented.

A

Index		Day	Control	Fraction B of *Caltha palustris* extract		
				1 × 10 mg/kg	1 × 1 mg/kg	1 × 0.1 mg/kg
$CD3^+$	(%)	1	48.00 ±2.92	54.04 ±3.05*	53.83 ±4.58*	53.36 ±5.01*
	($\times 10^6$)		2.32 ±0.93	4.34 ±2.73	3.90 ±1.83*	5.83 ±1.97*
	(%)	3	50.19 ±5.30	53.19 ±6.32	53.76 ±4.02	57.31 ±2.98
	($\times 10^6$)		9.19 ±2.12	8.70 ±2.37	7.20 ±0.80*	9.55 ±1.51
$CD4^+$	(%)	1	36.99 ±10.72	45.81 ±1.89*	44.90 ±2.53*	42.92 ±3.88
	($\times 10^6$)		1.75 ±0.90	3.64 ±2.25	3.28 ±1.59*	4.47 ±1.56*
	(%)	3	43.36 ±3.52	44.45 ±4.13	43.88 ±1.88	47.17 ±2.79*
	($\times 10^6$)		7.87 ±1.53	7.27 ±1.97	5.54 ±0.79*	7.87 ±1.33
$CD8^+$	(%)	1	6.00 ±1.85	8.96 ±0.81	9.83 ±1.79	8.73 ±1.08
	($\times 10^6$)		0.29 ±0.15	0.75 ±0.51	0.7 ±0.3*	0.97 ±0.36*
	(%)	3	7.40 ±0.64	7.21 ±0.24	7.42 ±0.94	8.79 ±0.31*
	($\times 10^6$)		1.36 ±0.33	1.16 ±0.19	0.95 ±0.16*	1.46 ±0.17
$CD19^+$	(%)	1	49.51 ±3.21	43.87 ±3.27*	44.02 ±4.59*	44.67 ±5.10*
	($\times 10^6$)		2.35 ±0.69	4.81 ±3.63	3.34 ±1.86	4.89 ±1.99*
	(%)	3	43.96 ±3.3	42.94 ±6.43	42.72 ±4.21	40.63 ±3.06*
	($\times 10^6$)		7.98 ±1.66	6.84 ±0.91	5.42 ±0.70*	6.69 ±0.38*

B

Index		Day	Control	Fraction B of *Caltha palustris* extract		
				5 × 10 mg/kg	5 × 1 mg/kg	5 × 0.1 mg/kg
$CD3^+$	(%)	1	54.97 ±5.67	60.39 ±4.57	52.07 ±3.21	58.57 ±7.40
	($\times 10^6$)		6.00 ±1.16	2.66 ±1.53*	2.67 ±0.78*	3.42 ±1.11*
	(%)	3	53.31 ±6.48	45.16 ±6.97*	29.84 ±10.57*	41.20 ±2.87*
	($\times 10^6$)		8.86 ±3.29	4.06 ±1.59*	1.97 ±0.57*	2.95 ±0.37*
$CD4^+$	(%)	1	43.42 ±5.81	44.50 ±1.19	41.66 ±3.05	46.31 ±5.30
	($\times 10^6$)		5.04 ±1.10	1.96 ±1.09*	2.15 ±0.68*	2.73 ±0.92*
	(%)	3	45.05 ±3.74	37.35 ±8.39*	26.83 ±9.44*	35.11 ±1.63*
	($\times 10^6$)		6.44 ±1.17	4.08 ±0.83*	1.74 ±0.58*	1.38 ±0.70*
$CD8^+$	(%)	1	7.80 ±1.42	10.10 ±2.57*	7.44 ±1.70	10.58 ±2.47*
	($\times 10^6$)		0.89 ±0.26	0.42 ±0.20*	0.38 ±.014	0.62 ±0.25
	(%)	3	7.48 ±1.08	7.08 ±1.66	3.50 ±1.18*	4.69 ±0.31*
	($\times 10^6$)		1.23 ±0.39	0.64 ±0.28*	0.22 ±0.07*	0.34 ±0.03*
$CD19^+$	(%)	1	44.03 ±7.52	37.54 ±4.11	44.80 ±3.64	39.35 ±7.53
	($\times 10^6$)		4.80 ±1.87	1.62 ±0.89*	2.37 ±0.82*	2.40 ±1.09*
	(%)	3	44.39 ±6.31	52.34 ±7.29*	67.67 ±10.2*	56.67 ±3.11*
	($\times 10^6$)		8.14 ±4.48	4.85 ±1.95	4.10 ±1.32*	4.16 ±4.82

**p < 0.05 as compared to the control group*

T-lymphocyte in peripheral blood of tumor-bearing mice. In our study, a significant decrease in the percentage of single-positive $CD8^+$ thymocytes and splenocytes after administration of fraction B from *Caltha palustris* extract was found.

Our study showed that a single administration of examined fraction B decreased the $CD19^+$ B lymphocyte population in mesenteric lymph nodes while multiple administration decreased this parameter only temporarily. In turn, $CD19^+$ B lymphocyte population of spleen has been stimulated by five times administration of the tested agent. Han *et al.* [23] demonstrated in their studies the stimulating activity of polysaccharide extracts isolated from the radix of *Platycodon grandiflorum* (PG). *Platycodon grandiflorum* was found to markedly increase polyclonal IgM antibody production and the proliferation of B cells. Furthermore, an increase in IgM antibody production in B cells was demonstrated after the intraperitoneal administration of PG in mice immunized using T-dependent antigen, sheep red blood cells (SRBCs). Also Duradao *et al.* [24] demonstrated stimulating activity on splenic B cells in *in vitro* and *in vivo* test of polysaccharide fraction E extracted from *Prunus dulcis* seeds. The present study showed that fraction B from *Caltha palustris* extract is able to change the percentage and absolute number of $CD4^+$ T cell subpopulation in thymus, and $CD4^+$, $CD8^+$ cell subpopulations in spleen and B lymphocytes in the spleen and mesenteric lymph nodes. The effect of the investigated substance depended on the number of consecutive doses applied. Further research on the structure and relationship between the structure and immunomodulatory activities of the polysaccharides fraction B from *Caltha palustris* extract is required.

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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